

Using Gene Editing and an Accumulated Bioproduct as a Reporter for Genotypic and Phenotypic Heterogeneity in Growth-vs-Production for *Methylobacterium extorquens* Conversion of Aromatics to Butanol

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<https://marxlab.org/doe-biosystems-project/>

Project Goals: With a unique capacity to assay growth and production – for either a tremendous number of genotypes in a mixture, or for individual cells – we will provide an unprecedented view of the critical tradeoff between growth and production. This will be used to guide development of *M. extorquens* as a novel platform for conversion of methoxylated aromatics to butanol. We will accomplish this work through the following aims:

- 1. Engineer/evolve improved use of methoxylated aromatics in M. extorquens*
- 2. Explore growth-vs-production tradeoffs for genetic and phenotypic variation in PHB production*
- 3. Combine improvements in substrate use and production capacity*
- 4. Exchange PHB synthesis for butanol synthesis to test best genotypes*

Abstract. Over the past year we have made progress on several fronts in order to move towards achieving our project's goals. First, we have developed a novel "Hi-PIE" method to introduce of genome-edited gene clusters from *Vibrio* into *Methylobacterium*. Second, we have developed the capacity to visualize PHB in unstained cells of *M. extorquens*. Third, we have developed a chromosomal system for tracking gene expression of multiple loci in single cells of *M. extorquens*. Fourth, we have completed analysis of the novel pathway for aromatic utilization that we have found in *M. extorquens* and we have evolved strains to effectively utilize vanillic acid. Fifth, and perhaps most importantly, we have uncovered that utilization of methoxylated aromatics depends critically upon the ability to handle formaldehyde toxicity, and that this capacity involves a substantial degree of phenotypic heterogeneity. All of these steps forward move us toward our ultimate goal to develop *M. extorquens* for conversion of methoxylated aromatics to butanol.

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